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ABSTRACT

The large variation in individual response to infection with Rift Valley fever virus (RVFV) suggests that host genetic determinants play a role in determining virus-induced disease outcomes. These genetic factors are still unknown. The systemic inoculation of mice with RVFV reproduces major pathological features of severe human disease, notably the hepatitis and encephalitis. A genome scan performed on 546 (BALB/c × MBT) F2 progeny identified three quantitative trait loci (QTLs), denoted *Rvfs-1* to *Rvfs-3,* that were associated with disease susceptibility in MBT/Pas mice. Non parametric interval-mapping revealed one significant and two suggestive linkages with survival time on chromosomes 2 (*Rvfs-1*), 5 (*Rvfs-3*), and 11 (*Rvfs-2*) with respective LOD scores of 4.58, 2.95 and 2.99. The two-part model, combining survival time and survival/death, identified one significant linkage to *Rvfs-2* and one suggestive linkage to *Rvfs-1* with respective LOD scores of 5.12 and 4.55. Under a multiple model, with additive effects and sex as a covariate, the three QTLs explained 8.3 % of the phenotypic variance. Sex had the strongest influence on susceptibility. The contribution of *Rvfs-1*, *Rvfs-2,* and *Rvfs-3* to survival time of RVFV-infected mice was further confirmed in congenic mice.

INTRODUCTION

Rift Valley fever (RVF) is a serious emerging viral zoonosis that primarily affects ruminants and humans. Recurrent outbreaks have been documented in sub-Saharan Africa and have spread outside continental Africa to Madagascar, and the Arabian Peninsula, killing hundreds of thousands of animals and more than a thousand humans.[1,2] The RVF virus (RVFV), a member of the *Bunyaviridae* family, genus *Phlebovirus*, is mainly transmitted by mosquitos and causes necrotic hepatitis, hemorrhage and abortions with high mortality among newborn and young animals.[3] Humans can also be infected through aerosols or by physical contact with body fluids, and organs of infected animals.[4,5] Some reports indicated that the disease predominantly affects male patients,[6] which could result from increased occupational and professional contacts with animals. Most patients suffer a self-limiting, febrile illness. However, a subset of patients develop severe forms characterized by hepatitis with fatal hemorrhagic fever or neurological disorders. The mortality rate has been reported to vary from 1 to 14%.[7,8] Due to the possibility of acute disease and the ease of aerosolization of infectious viral particles, RVFV outbreaks and research are carefully monitored by government agencies to prevent its potential use in bioterrorism.[9]

Progress in molecular biology of RVFV has been made during the last decade.[10,11] The genome of RVFV consists of a single-stranded tripartite RNA (L, M, and S segments) of negative-sense or ambisense polarity. The L segment encodes the RNA-dependent RNA polymerase, while the M segment encodes the envelope glycoproteins (Gn/Gc) and the non-structural NSm protein. The S segment encodes the N nucleocapsid and the NSs nonstructural protein. The NSs protein is the major virulence factor counteracting both the antiviral interferon (IFN)-β response and the double-stranded RNA (dsRNA)-dependent protein kinase (PKR) activity.[12-14] Little is known about the natural host factors that influence the progression and severity of RVF disease in humans and animals. The large variation in individual response to the infectious agent suggests the existence of host genetic factors influencing susceptibility to RVF disease.[1,2] At present, it is impossible to dissect host genetic determinants of RVF severity in humans, or livestock, as no one has access to the large number of cases required for a genome-wide association study

(GWAS). Animal models of RVF disease are needed to perform functional studies in a controlled setting,[15] and to better define genetic susceptibility factors. The susceptibility of rats to RVFV differs among strains.[16-18] Previous experiments with the rat model indicated a single Mendelian gene with dominant inheritance in the resistance phenotype of WF/mol rats although the underlying polymorphism has not yet been identified.[19,20]

Mouse models have provided key insights into the biology of human diseases and paved the way for the development of novel therapeutic approaches.[21] We have previously shown that wild-derived inbred MBT/Pas (MBT) mice are the most susceptible to infection with RVFV virulent strains, while BALB/cByJ (BALB/c) mice show the highest resistance among the tested strains.[22] Following infection with the wild-type virus strain ZH548, MBT mice exhibited earlier and higher viremia compared to BALB/c mice. Interestingly, the susceptibility of MBT mice to RVF disease seems to be selective since these mice survived an infection with either influenza A virus or West Nile virus in conditions where BALB/c mice died.[22]

To investigate the inheritance of susceptibility to RVF disease, we used a genome-wide quantitative trait locus (QTL) search strategy. This method does not require prior hypotheses regarding the pathogenesis of the disease. The analysis of survival time identified three QTLs on chromosomes (Chr) 2, 5 and 11, designated Rift Valley fever virus susceptible locus (*Rvfs*)-1, -3 and -2 respectively. Chromosomal regions spanning the QTLs from the susceptible strain (MBT) were transferred into the resistant recipient (BALB/c) background. Each congenic strain (denoted C.MBT-*Rvfs*) carries a BALB/c genetic background with only the chromosomal segment encompassing the corresponding *Rvfs* locus from MBT. Phenotypic differences in the C.MBT-*Rvfs-1, -2* and *-3* congenic strains relative to BALB/c mice confirmed the effects of the three QTLs. Our data also support the existence of sex-specific genetic variants governing susceptibility to RVFV-induced disease.

RESULTS

Segregation analysis of the RVFV susceptible phenotype in BALB/c and MBT F2 cross

In a previous study, we identified the MBT inbred strain, an inbred derivative of wild caught animals, as highly susceptible to infections with virulent strains of RVFV. When infected intraperitoneally with 100 PFU of RVFV ZH548 strain, most MBT mice died within 3 days while BALB/c animals survived longer.[22] The difference of susceptibility between MBT and BALB/c mice indicates that host genetic factors control outcomes following infection with RVFV. In our experiments, MBT and BALB/c were considered as the susceptible and resistant strain, respectively. To investigate the mode of inheritance of MBT susceptibility to RVFV infection, we examined the mortality of 116 (BALB/c × MBT) F1 hybrids after RVFV infection (Figure 1A). Significant differences in sex-specific susceptibility were seen in F1 mice, as well as in parental BALB/c and MBT mice, the males were more susceptible than the females (log-rank test; $P<0.0001$ for BALB/c and F1; $P<0.001$ for MBT). The F1 population displayed similar survival curves as the BALB/c strain (log-rank test; $P=0.1490$ and 0.1381 for female and male mice, respectively), while they were significantly different from the MBT strain (log-lank test; $P<0.05$ for both female and male mice). The mean time-to-death in the F1 population (6.20±0.25 and 6.00±0.20 days for females and males) was significantly lower than that of BALB/c mice in both sexes (8.17±0.24 and 6.70±0.21 days for females and males, respectively; surviving animals were not included; one-way ANOVA with Tukey's post hoc test).

F2 progeny were produced, infected with the RVFV and monitored. The F2 population showed intermediate survival curves, significantly different from both parental strains (Figure 1A). Furthermore, the mean time to death of the F2 population showed a continuous distribution within the range of parental phenotypes, suggesting a multigenic control (Figure 1B). Male mice presented a shorter time to death and a higher mortality rate than females in F2 populations (log-rank test; $P<0.0001$).

Genetic dissection of the susceptible phenotype in BALB/c and MBT F2 intercross

To identify genetic components underlying the susceptibility to RVFV infection in the MBT mice, a whole genome scan was performed to evaluate 546 (BALB/c × MBT) F2 progeny. A total of 259 polymorphic markers located on all autosomal chromosomes and on the X chromosome were assayed. Out of 546 RVFV-infected mice, 41 individuals (7.5%) survived more than 9 days post-infection (p.i.). To map the QTLs, we applied methods developed by Broman which account for spikes in the phenotype distribution.[23] A binary analysis of survival/death with sex as a covariate failed to reveal any significant QTLs, probably due to the small number of surviving mice. We performed nonparametric interval-mapping with the time to death after infection as a trait. Surviving mice were excluded, leaving 505 non-surviving animals for analysis. One significant QTL was detected on Chr 2 with the peak LOD score reaching 4.58 at genomic position 168.2 Mb (P=0.005). Two suggestive QTLs were also found on Chr 5 (LOD=2.95; P=0.160 at genomic position 61.6 Mb) and Chr 11 (LOD=2.99; P=0.154 at genomic position 113.9 Mb) (Figure 2A). We also applied a two-part model, combining the binary trait (survival/death), and the quantitative trait (survival time)[23]. One significant and one suggestive QTLs were detected on Chr 11 (LOD=5.12; P=0.022 at genomic position 112.8 Mb) and Chr 2 (LOD=4.55; P=0.075 at genomic position 168.2 Mb), respectively (Figure 2B). No significant QTLs were detected when these QTL tests were performed on females and males separately. Of note, the loci on Chr 2 and 11 achieved the 5% genome-wide significance level only with the nonparametric interval-mapping and the two-part model, respectively. No QTLs were found on X chromosome which could have contributed to the sex difference in susceptibility.

We then investigated the genetic interactions (additive or epistatic) between the three QTLs, using the scantwo command of R/qtl (Figure 3). This analysis provided evidence for significant additive effects between Chr 2 and 11 (LOD=6.86; P=0.014), and between Chr 2 and 5 (LOD=6.73; P=0.016). A suggestive additive effect was also detected between Chr 5 and 11 (LOD=5.85; P=0.089). There was no evidence for epistatic interactions. To estimate the effect of each QTL on the phenotype, we fitted a multiple-QTL model under the hypothesis that the three

QTLs on Chr 2, 5, and 11 contribute additively to the susceptibility to RVFV infection (as measured as the time to death) using sex as a covariate. As a result, we found that the QTLs collectively explain 8.3% of the phenotypic variance in the F2 population whereas sex had the strongest effect on the phenotype, explaining 10.1% of the variance (Table 1). The three QTLs will be referred to as *Rift Valley fever susceptible locus*-1 (*Rvfs1*) on Chr 2, *Rvfs2* on Chr 11 and *Rvfs3* on Chr 5. The confidence intervals, based on two-LOD units drop-off from the QTL peaks, were determined by the markers *D2Mit306* and *rs3664044* for *Rvfs1*, *rs13481186* and *D11Mit69* for *Rvfs2*, and *D5Mit125* and *rs4225536* for *Rvfs3* (Table 1).

Derivation and susceptibility of congenic strains

Congenic strains were generated in order to confirm the individual effects of *Rvfs1*, *Rvfs2* and *Rvfs3* on the susceptibility to the RVFV infection. The critical interval for each of the QTLs was transferred from the MBT genome onto a BALB/c genetic background by at least ten cycles of backcrossing using marker-assisted introgression. Two heterozygotes were then intercrossed and two homozygous offspring were bred to fix the MBT haplotype of *Rvfs1*, *Rvfs2* and *Rvfs3* on the BALB/c background, resulting in the C.MBT-*Rvfs1*, C.MBT-*Rvfs2* and C.MBT-*Rvfs3* congenic strains. To characterize precisely the introgressed segments, congenic mice were analyzed using the MegaMUGA platform that includes 77.8K markers.[24] The analysis revealed that the MBT segment on Chr 2 in C.MBT-*Rvfs1* spans from a point between 2:164,791,844 and 2:164,836,539 to the end of chromosome. Similarly, the MBT segment on Chr 11 in C.MBT-*Rvfs2* spans from a point between 11:104,823,629 and 11:104,845,860 to the end of chromosome, and on Chr 5 in C.MBT-*Rvfs3* spans from a point between 5:53,334,387 and 5:53,365,815 to a point between 5:120,706,275 and 5:120,737,326 (Figure 4). No unlinked MBT markers were found in the congenic genomes, confirming that only the *Rvfs* chromosomal segments were introgressed onto the BALB/c genetic background.

The three congenic strains were challenged with 100 PFU of RVFV ZH548 strain. In the C.MBT-*Rvfs2* and C.MBT-*Rvfs3* strains, the males were more susceptible than the females (log-rank test; $P<0.001$ and $P<0.0001$ respectively). Difference in sex-specificity was not significant in C.MBT-*Rvfs1* mice using cohort sizes of 16 females

and 20 males. C.MBT-*Rvfs1* females died significantly earlier than BALB/c females, while no significant difference was observed between C.MBT-*Rvfs1* and BALB/c males (Figure 4A). C.MBT-*Rvfs2* mice of both genders died significantly earlier than BALB/c mice (Figure 4B). Finally, C.MBT-*Rvfs3* males died significantly earlier than BALB/c males. These results confirmed the effects of the three QTLs on the susceptible phenotype. Unexpectedly, however, C.MBT-*Rvfs3* females survived significantly longer than BALB/c females, an unexpected result which would require confirmation on a larger cohort (Figure 4C). No significant differences were observed between BALB/c mice and animals heterozygous for the haplotypes of *Rvfs1*, *Rvfs2* or *Rvfs3.* This indicates that these QTLs have recessive effects while on the BALB/c genetic background.

DISCUSSION

Host genetic control of RVF disease in mice

Susceptibility to infectious disease is difficult to assay in humans, and human GWAS would require tens or hundreds of thousands samples of RVFV-infected patients. Mice provide an alternative means of studying RVF disease since they recapitulate the acute-onset hepatitis and delayed-onset encephalitis seen in severe human RVF.[25] The current study was designed to assess the effect of polymorphisms in the mouse genome on survival after infection with a virulent strain of the RVFV. Since previous attempts to identify an influence of genetic factors in classical laboratory strains have failed,[20] we tested the susceptibility of inbred strains derived from progenitors of various *Mus* subspecies, and found that mice of the *Mus m. musculus* derived MBT strain exhibited an extreme susceptibility to RVFV infection.[22] We show here that the susceptibility of MBT mice to RVFV infection is a complex trait that is inherited in a multifactorial fashion with sex influencing the severity of infection. We identified three host genome regions associated with severity of RVF disease in inbred mice. Each of these QTLs explains between 1.78% and 3.17% of the phenotypic variance. These results are consistent with data from a comprehensive analysis of the genetic architecture of behavioral and physiological phenotypes in the mouse which indicate that most QTLs explain between 1% and 5% of the genetic variance, and only 1% of the QTLs have an effect greater than 5%.[26] QTLs with larger effect sizes

on survival time following infection were occasionally identified.[27,28] Altogether, only 8.3% of the phenotypic variance in the (BALB/c × MBT)F2 could be ascribed to genetic determinants meaning that there must be many undetected loci. This suggests that susceptibility to RVFV infection is controlled by many variants of modest effect.

We have previously shown that mouse embryonic fibroblasts derived from MBT mice lacked a complete type I IFN response, suggesting that inappropriate innate immune response could account for the susceptibility of MBT mice.[22] A search for candidate genes using PosMed program[29] with "innate immune response" as a keyword gave a list of 69, 105, and 180 genes for the critical interval as defined by C.MBT-*Rvfs1,* -*Rvfs2* and -*Rvfs3* congenic strains respectively*.* This high number of candidate genes was expected given the large size of the critical regions, 16.9 Mb, 16.9 Mb, and 67.4 Mb, respectively. Therefore, the identification of the genes underlying the QTLs will first require the production of subcongenic strains for fine mapping to reach manageable numbers of candidate genes. Analysis of sequence variation between MBT and BALB/c genomes will also be performed. A drawback of using a wild-derived inbred strain is that the average divergence of approximately 1 SNP per 120 bp between *M. m. musculus* derived and laboratory genomes is higher than that observed between pairs of laboratory strains (1 SNP per 700 bp).[30] This higher number of naturally occurring but functionally neutral polymorphic variants will complicate the identification of the causal mutation. Gene expression profiling in the target tissues of RVF disease from RVFV-infected C.MBT-*Rvfs* congenic and BALB/c control mice will also be of great utility. This combined effort will help narrow *Rvfs* QTLs to testable lists of candidate genes.

Although this study is the first of its kind in the mouse, detailed analyses of RVFV-infected inbred rats also demonstrated that the host genotype determines the outcome of RVF disease. First evidence suggesting variation among rodent inbred lines came from Peters and co-workers who showed that Wistar-Furth (WF) inbred rats are highly susceptible to liver necrosis induced by subcutaneous infection with RVFV, while Lewis (LEW) rats are largely resistant.[17] Resistance to hepatitis in LEW rats was shown to be inherited as a simple Mendelian dominant trait,[20] and derivation of a congenic line confirmed that a single chromosomal

region was able to confer the resistance of LEW rats to the hepatic disease induced by infection with RVFV in susceptible WF rats.[19] The locus involved has not been further defined or mapped and thus its relationship to the QTLs defined in our study is unknown. Altogether, experiments in rodent models of RVFV infection support the hypothesis that the differences in pathogenesis between breeds of ruminants,[31,32,33] and among human patients[1] are in part the result of genetic factors that influence susceptibility to RVFV infection.

Effect of sex in susceptibility to RVF disease in mice

A variety of other factors can potentially influence the severity of RVF disease, e.g. the inoculation dose and virulence of the virus, the sex, and the immune status of the host. We observed a significant sex effect in mice, not only in the BALB/c and MBT parental strains, but also in the F1 and F2 populations, with enhanced susceptibility in males compared to females. In fact, sex was the main factor explaining variance in the F2, higher than all three QTLs together (Table 1). RVFV infection in mice demonstrated tropism for a variety of organs, including the ovaries.[25] Consistent with this, the ovaries and uterus in females, the seminal vesicles, preputial glands, epididymis and testis in males were identified as a site of viral replication in mice infected with a recombinant RVFV expressing the *Renilla reniformis* luciferase.[34] In rats, males were reported to be somewhat more susceptible than females, and castrated males were more resistant than intact rats.[20] This suggests the implication of sex hormones on the regulation of genes that underlie resistance to RVF disease in rodents. This is in line with our finding that two of the three *Rvfs* QTLs have stronger effect in one of the sexes, in females for *Rvfs1,* and in males for *Rvfs3*.

In humans, reduced susceptibility to viral infections among females have been reported, presumably because females mount higher immune responses than males.[35] However, lower female mortality due to infectious diseases is not universal, and a reverse pattern of excess female mortality has been observed for measles and influenza.[36,37] With regard to RVF disease, epidemiological studies in Saudi Arabia, Kenya, South Africa, Sudan, and Gabon indicate that men are more frequently infected by RVFV than women.[6,8,38-42] Direct contact with animal tissues, blood or other body fluids was reported to be the most common risk factor

and route of transmission of RVFV to humans in South Africa and Kenya.[38,39] Therefore enhanced male morbidity in these regions of Africa may be explained by the occupation of herding, which implies slaughtering and milking, predominantly performed by men.[43] Infected men were also reported to be at higher risk than women to develop severe RVF disease and eventually die.[38,43] Again, this may be the result of more frequent exposures to infected animals and their body fluids which provide opportunities for infection with large inocula of RVFV compared to the low number of infectious viral particles transmitted by bites from infected mosquitoes.[44] It is however not excluded that the higher fatality ratio measured in male human patients has also a biological basis as seen in rodents. Further clinical and epidemiological investigations in humans and genetic studies in rodents are needed to understand these sex and/or gender differences.

MATERIALS AND METHODS

Mice and crosses

BALB/cByJ (BALB/c) mice were purchased from Charles River Laboratories. The MBT/Pas (MBT) strain, derived from *M. m. musculus* progenitors trapped in Bulgaria in 1980,[45] is maintained at the Institut Pasteur. Female BALB/c mice were mated with male MBT mice to produce F1 hybrids. (BALB/c × MBT) F1 mice were intercrossed to produce F2 progeny (n=546) for the genome scan. To develop congenic strains, female BALB/c mice were crossed with MBT male mice. F1 females were backcrossed to BALB/c males and the BC1 progeny were genotyped for several polymorphic microsatellite markers within the QTL regions on Chr 2, 5 or 11. Males that carried heterozygous alleles for one of the QTLs but not for the other two QTLs were selected as breeders for the next generation. By the fifth generation, all breeders were heterozygous for the MBT alleles in the targeted region but homozygous for the BALB/c alleles in the other two QTL chromosomal segments. After reaching the tenth generation, mice were intercrossed to obtain homozygous animals. All F2 and congenic mice carried mitochondria and Y chromosome from BALB/c and MBT inbred strain respectively. Experiments on live mice were conducted according to the French and European regulations on care and protection of laboratory animals (EC Directive 86/609, French Law 2001-

486 issued on June 6, 2001) and the National Institutes of Health Animal Welfare (Insurance #A5476-01 issued on July 2, 2007). Experimental protocols were approved by the Animal Ethics Committee #1 of the *Comité Régional d'Ethique pour l'Expérimentation Animale* (CREEA), Ile de France (N°2012-0025), and carried out in compliance with Institut Pasteur Biosafety Committee.

Virus production and mouse infection

The RVFV strain ZH548, isolated from a male patient with the acute febrile illness at Zagazig fever hospital, Egypt (obtained from Centre National de Référence des Fièvres Hémorragiques Virales, Institut Pasteur, Lyon, France),[46,47] was used for all infection studies. Virus was titrated by plaque assay on monolayers of Vero E6 cells.[48] Mice were bred under specific pathogen free conditions and were transferred one week before infection into a BSL-3 isolator to allow acclimatization. Groups of 9- to 13-week-old animals were infected intraperitoneally with 100 PFU of RVFV strain ZH548. Morbidity, mortality and clinical disease scores were monitored daily for 14 days following infection. Animals that survived were euthanized on the last day of the monitoring period. Survival curves of congenic animals represent the pooled data from four to twelve experiments.

Genotyping and QTL mapping

Tail biopsies were collected at weaning from F2 progeny and high-quality DNA was prepared by standard phenol-chloroform extraction. Genotyping of F2 mice was performed using the GoldenGate platform (Illumina Inc.). A total of 484 SNPs were genotyped. Out of them, 244 markers were polymorphic and gave reliable genotypes, covering the entire mouse genome. After the first analysis, 15 polymorphic microsatellite markers in the QTL regions were added to better define those regions. An interval mapping for the survival phenotype was performed with the R/qtl software (version 2.15.1) for mapping quantitative trait loci under a non-parametric model (death/survival) or under the two-part model (death/survival, and survival time).[23,27,49] Genome-wide thresholds for significance were determined by a 1,000-permutation test. QTL were considered to be significant when the LOD scores exceeded the 5% genome-wide threshold and suggestive when the LOD scores exceeded the 20% genome-wide threshold.

All significant and suggestive QTLs were assessed in a multiple-QTL model with sex as a covariate (formula=y~sex+Q1+Q2+Q3), using makeqtl and fitqtl functions in R/qtl, and the percentage of phenotypic variance was estimated. The 2-LOD units drop-off was used to estimate the 95% confidence interval of each QTL. Tail DNAs of congenic strains were collected and analyzed with the MegaMUGA genotyping microarray (Neogen/Geneseek, Lincoln, NE), a new 78,000-probe array based on the Illumina® Infinium platform.[24] Markers on the MegaMUGA are distributed genome-wide with an average spacing of 33 kb.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad). Survival curves were compared by log-rank test.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Analysis in a multiple QTL model with sex as a covariate

	Position (Mb)	Nearest marker	LOD	% variance explained	P-value	2-LOD support interval	Locus
Sex			12.8	10.10	2.41e-14		
Chr 2	168.2	*rs27311433*	4.2	3.17	7.32e-05	*D2Mit306 - rs3664044*	*Rvfs1*
Chr 11	113.9	*D11Mit214*	3.8	2.88	0.00017	*rs13481186 – D11Mit69*	*Rvfs2*
Chr 5	61.6	*rs13478310*	2.3	1.78	0.00452	*D5Mit125 – rs4225536*	*Rvfs3*

FIGURE LEGENDS

Figure 1. Survival time of BALB/c, MBT, F1, and F2 mice after infection with RVFV ZH548 strain. (A) Nine- to twelve-week-old BALB/c, MBT, F1, and F2 female and male mice were infected intraperitoneally with 100 PFU of the RVFV ZH548. The survival of individual female (top) and male (bottom) mice was monitored until day 10 post-infection. Kaplan-Meier survival plots were recorded (log-rank test; the number of animals per genotype is given within brackets; *, $P<0.05$; **, $P<0.01$; ****, $P<0.0001$). (B) The distribution of F2 population and parental strains after infection with RVFV ZH548. Circles and triangles represent individual female and male mice, respectively. Depicted are the means and SD of female (n=43) and male (n=46) MBT mice, female (n=211) and male (n=360) F2 mice, and female (n=34) and male (n=40) BALB/c mice. Animals that survived more than 10 days post-infection were not included.

Figure 2. Genome-wide QTL scans for the outcome of RVFV infection in (BALB/c × MBT) F2 mice. A total of 546 F2 animals were genotyped with 259 polymorphic markers, and infected with RVFV ZH548 intraperitoneally. The survival of individual mice was monitored daily for a period of 14 days. (A) Nonparametric interval-mapping with the time to death as a trait, includes the 505 mice that died. LOD score is plotted as a function of genomic position. One significant locus on chromosome 2 and two suggestive loci on chromosomes 5 and 11 are revealed. (B) Two-part model combining the time to death and the binary trait (survived/died) of all 546 F2 animals. The large red dotted line represents the LOD trace for the nonparametric analysis, the small blue dashed line represents the LOD trace for the analysis of the binary trait (survive versus death), and the continuous black line is the sum of the two separate analyses. One significant locus on chromosome 11 and one suggestive locus on chromosome 2 are found. The horizontal lines represent the genome wide significance thresholds after 1,000 permutations (full line, $P=0.05$; long dash, $P=0.1$; small dash, $P=0.2$).

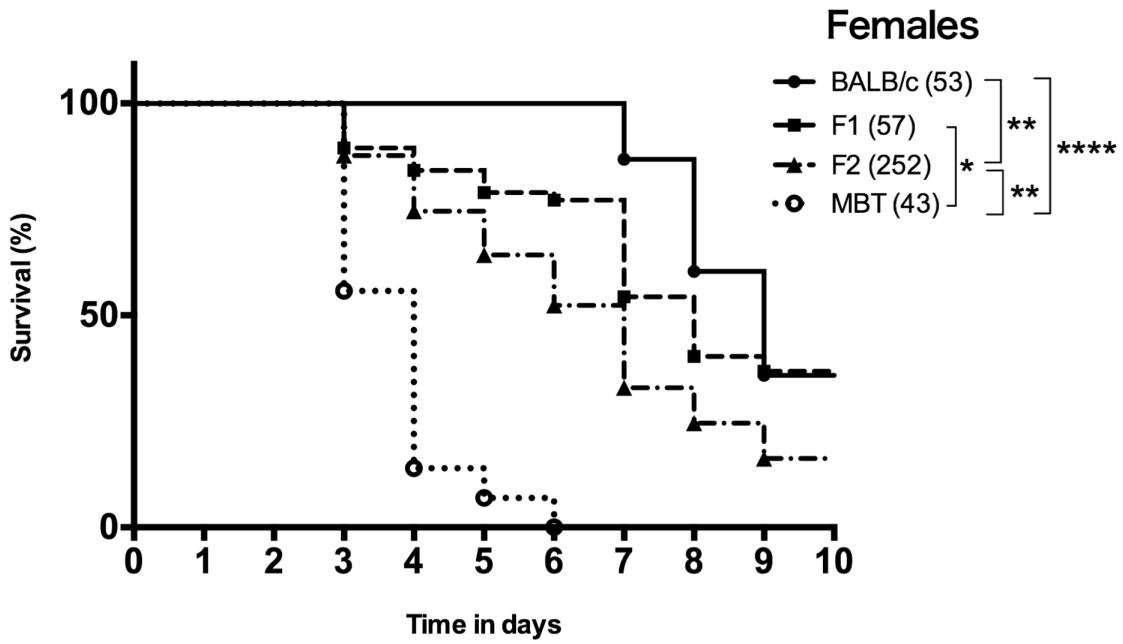
Figure 3. Close-up of two-dimensional genome scan on chromosomes 2, 5, and 11. The two-dimensional genome scan performed for the whole genome revealed additive effects between loci on chromosomes 2, 5, and 11. A close-up of the two-

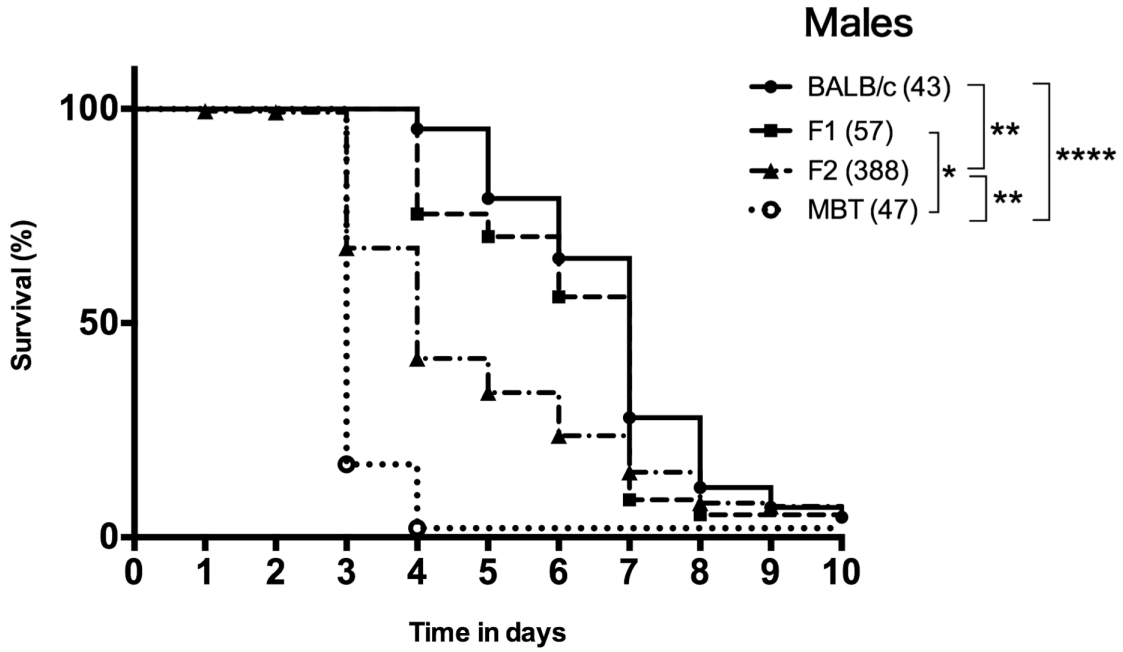
dimension scan on chromosomes 2, 5 and 11 is displayed with epistatic interaction in the lower right triangle, and additive effects in the upper left triangle. The color scale (right) indicates the additive LOD scores on the left and the interaction LOD scores on the right. The additive thresholds for significance levels 20% and 5% were calculated to be 5.25 and 6.15. The epistasis thresholds for the identification of novel interactions for significance levels 20% and 5% were 5.61 and 6.31. There is significant evidence in the upper left triangle for additive effects between loci on chromosomes 2 and 11, and between chromosomes 2 and 5, and suggestive evidence for additive effects between chromosomes 5 and 11. The results in the lower triangle indicate no significant evidence for epistatic interaction between chromosomes 2, 5, and 11.

Figure 4. Schematic representation of the MBT genomic regions fixed in the congenic strains and the congenic strain survival curves. (A) Haplotype structure of the congenic segment of chromosome 2 in C.MBT-*Rvfs1* (Rvfs1) congenic strain (left). The position of the MBT-derived chromosome 2 (grey) is shown on the BALB/c chromosome 2 background (black). Regions of unknown genotype are represented in white. Markers are identified along with their positions on the physical map (in bp). Kaplan-Meier survival plots of female and male Rvfs1 mice (right). The number of animals per genotype and gender is given within brackets. (B) Haplotype of the congenic segment of chromosome 11 in C.MBT-*Rvfs2* (Rvfs2) (left) and Kaplan-Meier survival plots of female and male Rvfs2 mice (right). (C) Haplotype of the congenic segment of chromosome 5 in C.MBT-*Rvfs3* (Rvfs3) mice (left) and Kaplan-Meier survival plots of female and male Rvfs3 mice (right). (log-rank test; *, $P<0.05$, ***, $P<0.001$).

Figure 1

A


Females
BALB/c (53)
F1 (57)
F2 (252)
MBT (43)
**
*
**
****
Survival (%)
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Time in days


Males
BALB/c (43)
F1 (57)
F2 (388)
MBT (47)
**
*
**
****
Survival (%)
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Time in days

B

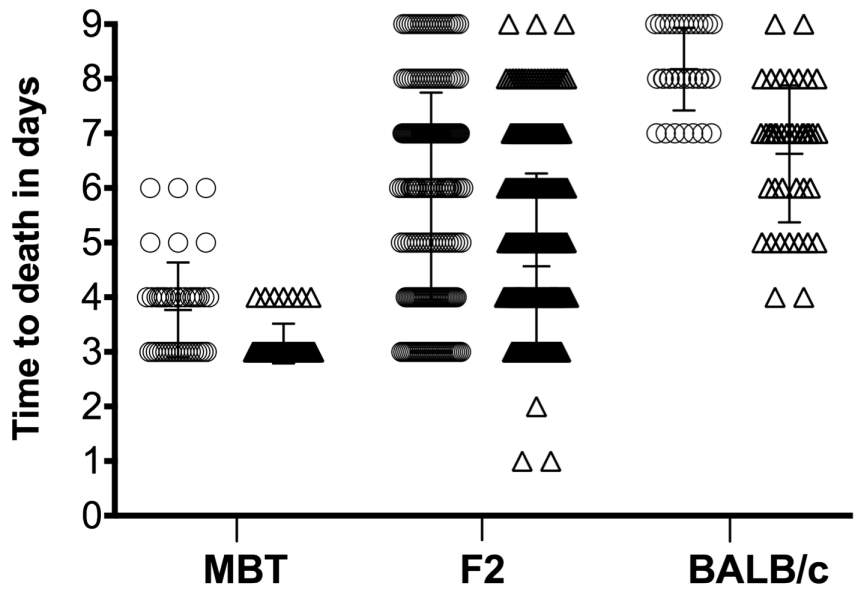

Time to death in days
9
8
7
6
5
4
3
2
1
0
MBT
F2
BALB/c

Figure 2

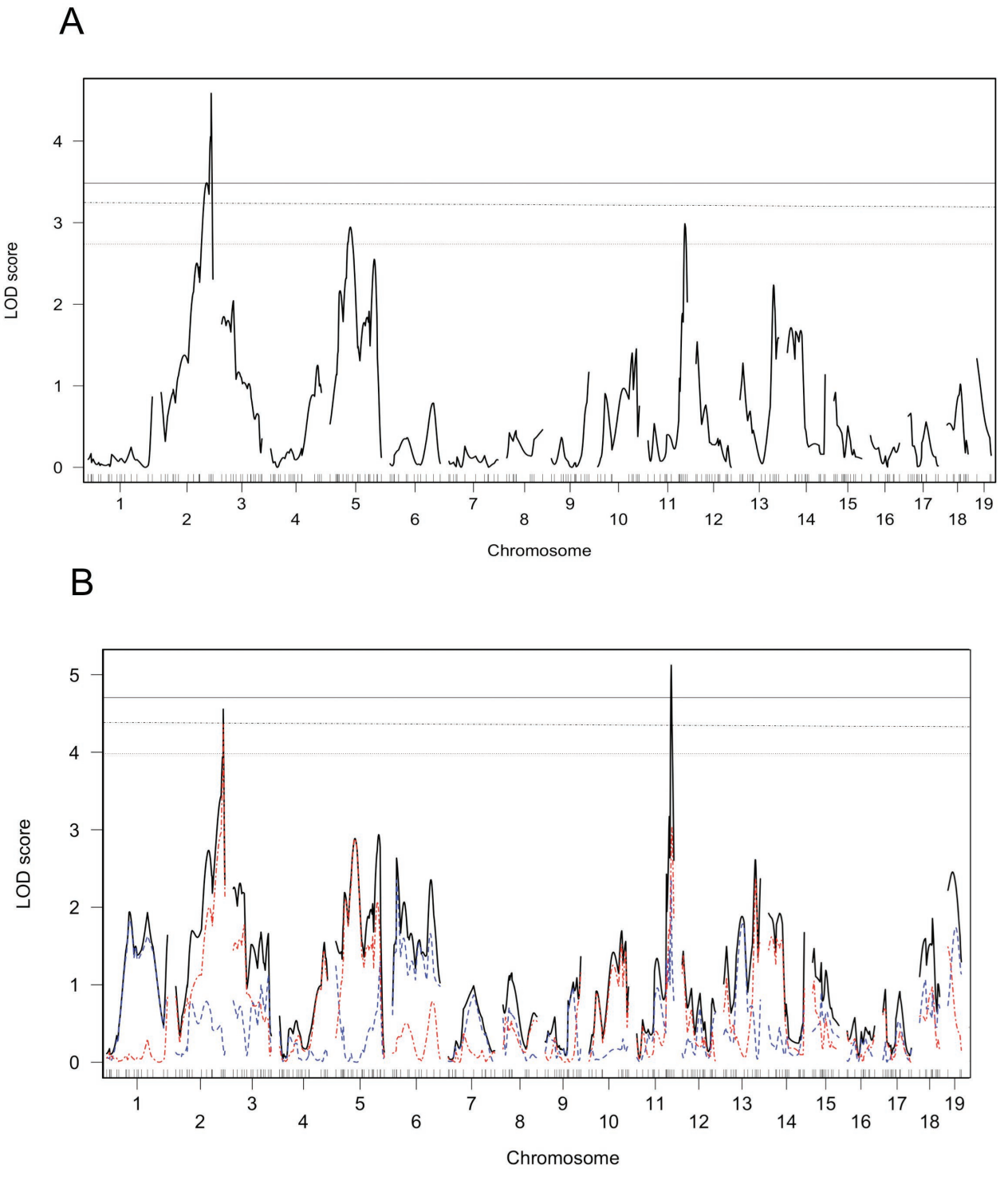

A
LOD score
0
1
2
3
4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Chromosome
B
LOD score
0
1
2
3
4
5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Chromosome

Figure 3

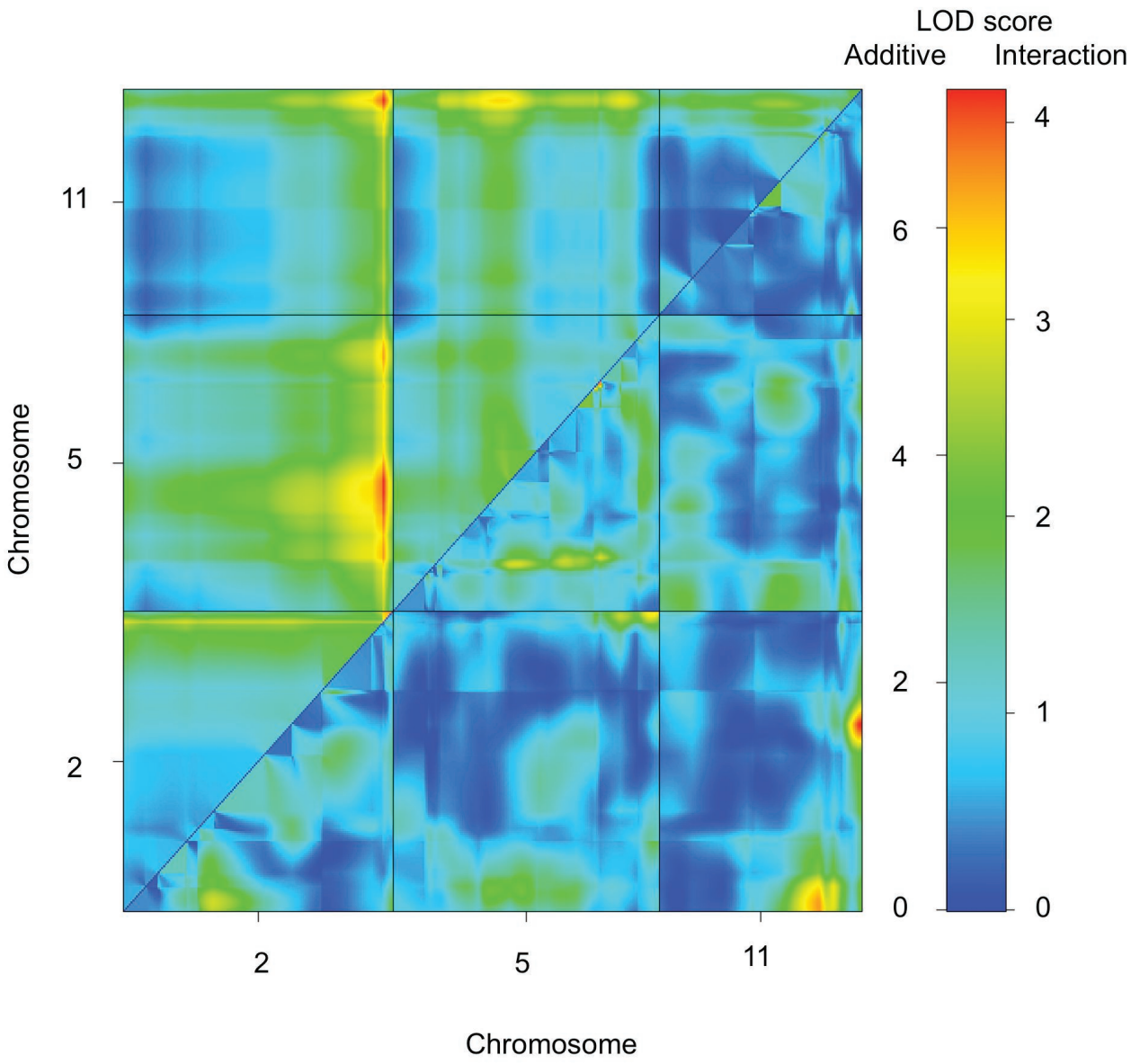
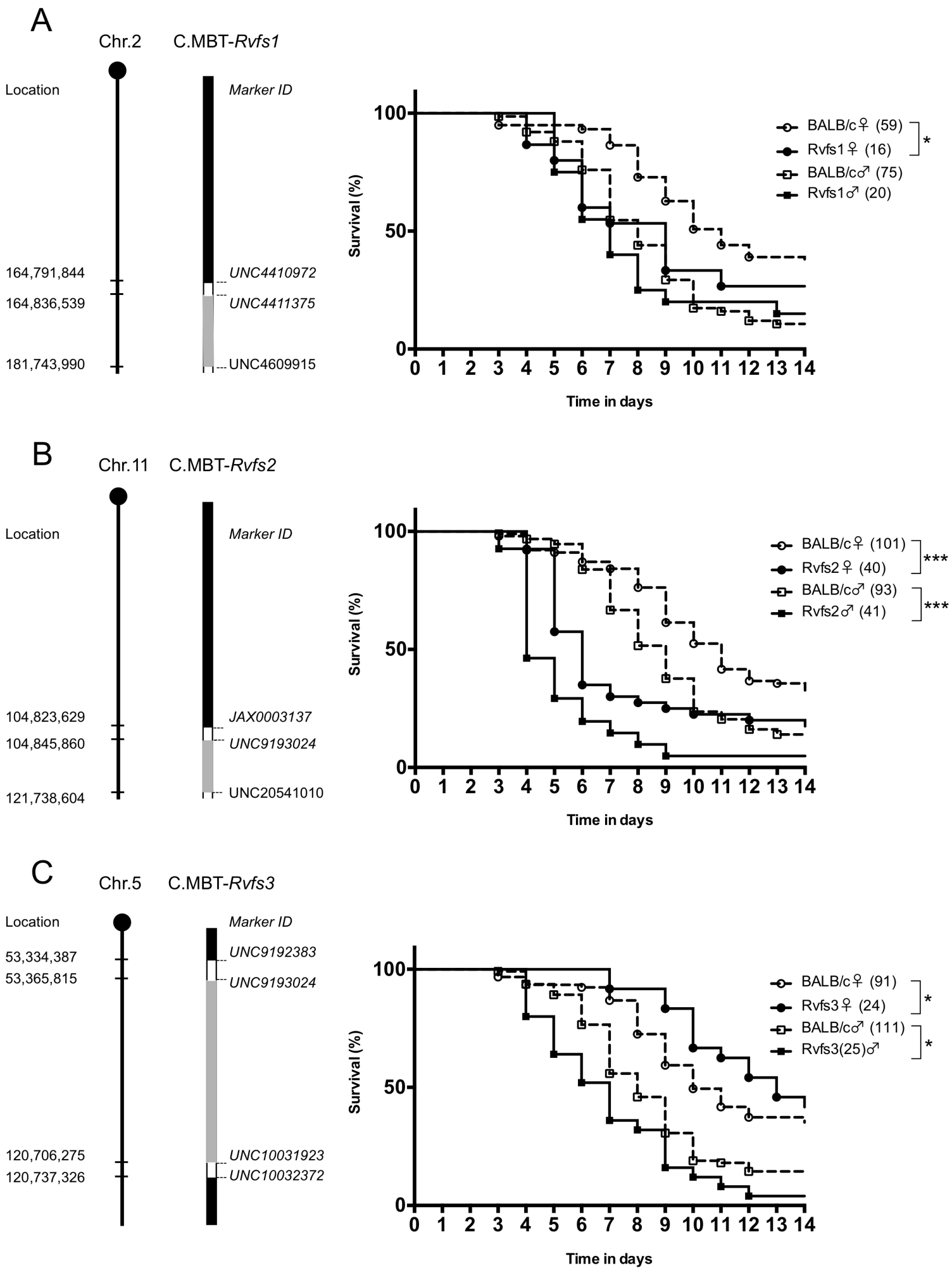

LOD score
Additive
Interaction
4
3
2
1
0
6
4
2
0
11
5
2
Chromosome
2
5
11
Chromosome

Figure 4


A
Chr.2 C.MBT-Rvfs1
Location
Marker ID
164,791,844
164,836,539
181,743,990
UNC4410972
UNC4411375
UNC4609915
Survival (%)
Time in days
BALB/c♀ (59)
Rvfs1♀ (16)
BALB/c♂ (75)
Rvfs1♂ (20)
*
B
Chr.11 C.MBT-Rvfs2
Location
Marker ID
104,823,629
104,845,860
121,738,604
JAX0003137
UNC9193024
UNC20541010
Survival (%)
Time in days
BALB/c♀ (101)
Rvfs2♀ (40)
BALB/c♂ (93)
Rvfs2♂ (41)
***
***
C
Chr.5 C.MBT-Rvfs3
Location
Marker ID
53,334,387
53,365,815
120,706,275
120,737,326
UNC9192383
UNC9193024
UNC10031923
UNC10032372
Survival (%)
Time in days
BALB/c♀ (91)
Rvfs3♀ (24)
BALB/c♂ (111)
Rvfs3(25)♂
*
*